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Evaluation of the Creatinine Assay on the Reflotron® Analyser under Centralized and Non-Centralized Conditions[1])

By *J. L. S. Dols* and *G. T. B. Sanders*

Academisch Medisch Centrum, Laboratorium voor Algemene Klinische Chemie, Amsterdam, The Netherlands

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Summary: The creatinine assay, performed on Reflotron® "dry chemistry" analysers in six offices of general practitioners, was evaluated as part of a project on the quality of laboratory determinations in decentralized situations. The method itself was evaluated before this investigation was started. Differences were noticed between Reflotron® machines, batches of strips, and various types of sample, viz. heparin-treated blood or plasma gave higher results than serum or EDTA-treated samples. All Reflotron® results were lower in comparison with wet chemistry methods. The "dry chemistry" technique was not influenced by the presence of glucose or bilirubin (below 170 μmol/l); haemoglobin interfered. By simultaneous sampling, all creatinine determinations performed on whole EDTA blood in the general practitioners offices were repeated on serum in centralized laboratories using "wet chemistry" techniques. In this comparison 3% of all creatinine determinations in general practitioners offices were outside the 95% confidence interval. We conclude that the creatinine determination on the Reflotron® may be used in general practitioners office testing as long as the general practitioner is aware of its limitations and can rely on a "reference" laboratory for training, quality control and repetition of unexpected results.

Introduction

The creatinine assay on the Reflotron® "dry chemistry" analyser is based on an enzymatic breakdown of creatinine followed by measurement of the liberated hydrogen peroxide (1). The reagents are applied in a dry form on a solid matrix. This so-called "dry chemistry" reagent system is also intended for use in decentralized testing, thus enabling a general practitioner to perform determinations in his/her office. The study described here is part of a greater evaluation of the influence of the introduction of facilities for chemical and simple haematological determinations on general medical practice. This article reflects the performance of the creatinine determination only, with special emphasis on the use of the method by non-laboratory skilled personnel. For this purpose we placed Reflotron® systems in general practitioners offices in two areas in the Netherlands. We compared the decentralized Reflotron® creatinine values with the results of Reflotron® determinations performed by laboratory technicians using "wet chemistry" measurements in two centralized ("reference") hospital laboratories, and with the results of a reference method for creatinine determination (based on the *Fuller*'s earth technique) (2, 3). In one of the central laboratories we performed all other tests associated with the evaluation of the performances of the creatinine determination on the Reflotron®.

Materials and Methods

Project setup

The project was carried out in two regions of the Netherlands, around the cities of Amsterdam (A) and Leeuwarden (L). The participants were six general practices and two central laboratories in the aforementioned cities, which served the general practitioners in their region. The distance between central lab-

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oratory and general practice was never more than 50 km. For more than 12 months the general practitioners used a Reflotron® 74F "dry chemistry" analyser (Boehringer Mannheim, Germany) and a CBC-5 haematology analyser (Coulter Electronics, Mijdrecht, The Netherlands), both connected to an Olivetti M250 computer (Olivetti, Leyden, The Netherlands). Identical analytical systems were installed in each central laboratory. The Reflotron® was used for the measurement of creatinine as one of the chemistry determinations performed by the general practitioners or his/her assistant; the relevant haematological quantities were determined on the CBC-5. The computer was used for a number fo purposes: for communication with the central laboratory, to collect all data of determinations on Reflotron® and CBC-5, and to control their quality. This latter aspect was assured by the software built in the computer, since, before Reflotron® or CBC-5 could be used for measurement of a specific test in patient samples, a control serum or blood had to be analysed. Only when this result fell within predefined limits, was the apparatus released for 24 hours for that test. Moreover, in the laboratory of the University Hospital of Amsterdam two extra Reflotron® instruments (Reflotron® 1 and 2) were used for test purposes.

Creatinine methods and reagents

Creatinine in the central laboratories was determined on a SMAC (Technicon, USA) (Amsterdam) and a Hitachi 717 (Boehringer Mannheim, Germany) (Leeuwarden) analyser, respectively. On both instruments reagents of their respective manufacturers were used. As a reference method for creatinine the technique with *Fuller*'s earth was used. On the Reflotrons® we used one lot number of Reflotron® creatinine reagent carriers with creatininase (Boehringer Mannheim product number 886874): 23462741; for checking differences between lots we used a second batch with lot number 23463232.

Centralized laboratories

Agreement between the results of the wet chemistry analysers in the two "reference" laboratories was checked with patient samples that were exchanged between the two locations. One location (Amsterdam) used heparin plasma, while the other (Leeuwarden) used serum in its normal routine setting. To compare results from both locations, three batches (each consisting of 30 heparin samples from Amsterdam and 10 serum samples from Leeuwarden) of normal routine samples were dispatched immediately after analysis to the other laboratory to be analysed directly after arrival. This was done on three separate days within a period of seven weeks. One sample (heparin) was of insufficient quantity and was omitted from the calculations. In total 119 samples were compared.

Blood samples

EDTA, serum and heparin samples were taken by venipuncture using evacuated blood collecting tubes (Venoject, Terumo Europe N. V., Leuven, Belgium). In the general practices blood samples were drawn from outpatients: EDTA blood for the in-office Reflotron® and CBC-5 determinations, and serum for the determinations in the two "reference" laboratories. All analyses in the general practitioners offices were performed with EDTA blood immediately after the samples were collected; the serum samples, collected at the same time, were sent as soon as possible to the "reference" laboratory in the region for comparison with the "wet chemistry" method for creatinine. Samples for the Reflotron® performance studies in one of the central laboratories (Amsterdam) were obtained from hospital patients and laboratory personnel (EDTA and heparin samples).

Controls

For quality control of the creatinine determinations in the laboratories and in the general practitioners offices we used several batches of precinorm U (analysed) control serum from Boehringer Mannheim, especially made for use on the Reflotron®. In the software of the computer, used for the 24 hour release of the Reflotron®, we applied the control limits as provided by the manufacturer.

Interfering substances

To evaluate the influence of some substances known to interfere with the creatinine determination, we mixed heparin plasma samples containing known high and low concentrations of glucose, bilirubin or haemoglobin, so as to obtain a full concentration range of each interfering component. Creatinine concentrations were then determined on the laboratory Reflotron® 1, the SMAC and with the *Fuller*'s earth method.

Statistical methods

For comparison of the creatinine results we evaluated the resulting data, following the statistical recommendation of *Passing & Bablok* (4, 5). For this purpose we used a software package called "EVAL-KIT" (6). For the interpretation of the results of the regression equations and the statistics on patient sample data we used the EP9-P guideline (7); outliers in the patient sample data of table 1e were determined as described in this guideline. The difference plot was created as described by *Pollock* et al. (8).

Results

Comparison of central laboratories

At the start of this study it was essential to have an insight into possible differences between the creatinine results of the two central laboratories. Table 1a shows the correlation data of the 119 samples. Based on the confidence interval ($p < 0.05$) for slope and intercept,

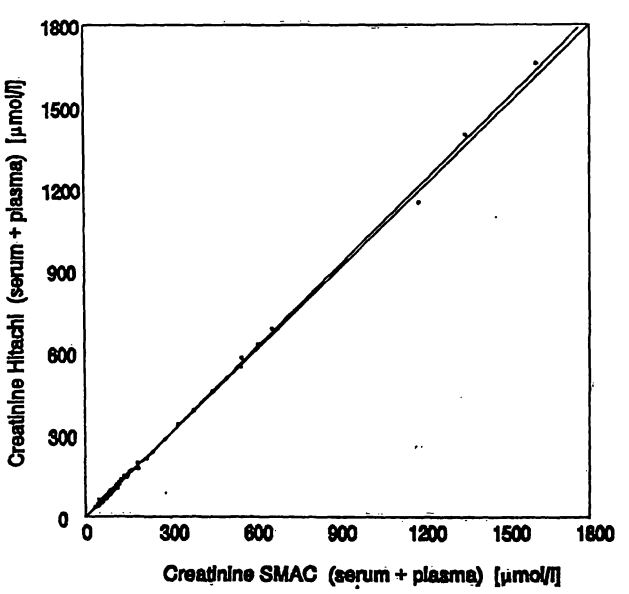

Fig. 1. Scatter plot and regression line of creatinine determinations in plasma and serum samples at the "reference" laboratories.

Tab. 1. Regression equations.

Method x	Method y	Slope	Intercept	r	r^2	Mean x	Mean y	N	Confidence interval	
									Slope	Intercept
a) Comparison between different instruments in the "reference" laboratories										
SMAC plasma	Hitachi plasma	1.026	1.212	0.999	0.998	164.8	168.9	89	1.006–1.035	0.44–2.80
SMAC serum	Hitachi serum	0.993	1.026	0.999	0.989	122.6	123.4	30	0.925–1.026	−2.01–7.59
SMAC serum + plasma	Hitachi serum + plasma	1.015	1.146	0.999	0.989	154.1	157.4	119	1.000–1.029	−0.22–2.70
b) Comparison of two batches of reagent strips										
Batch 23463232	Batch 23462741	1.053	4.733	0.924	0.854	73.7	81.5	46	0.931–1.200	−7.10–14.30
c) Use of different sample materials										
EDTA-blood Reflotron®	EDTA-plasma Reflotron®	1.067	−3.067	0.963	0.927	69.1	70.8	111	1.000–1.143	−7.91–1.00
EDTA-blood Reflotron®	Heparin-plasma Reflotron®	1.224	−2.322	0.945	0.893	69.1	83.3	111	1.167–1.328	−7.84–3.25
EDTA-plasma Reflotron®	Heparin-plasma Reflotron®	1.139	2.189	0.951	0.904	70.8	83.3	111	1.063–1.229	−2.99–7.82
EDTA-blood Reflotron®	Heparin-plasma SMAC	1.394	−7.808	0.910	0.828	69.1	86.2	111	1.286–1.512	−15.3–1.29
EDTA-plasma Reflotron®	Heparin-plasma SMAC	1.241	−0.296	0.928	0.861	70.8	86.2	111	1.158–1.333	−6.00–5.45
Heparin-plasma Reflotron®	Heparin-plasma SMAC	1.105	−4.184	0.951	0.904	83.3	86.2	111	1.042–1.171	−10.4–0.75
d) Comparison of Reflotron results with other techniques										
Fuller's earth	SMAC	0.892	4.309	0.999	0.998	153.4	140.2	78	0.878–0.901	2.77–5.68
Fuller's earth	Reflotron®	0.837	5.372	0.998	0.996	143.4	135.6	78	0.810–0.970	3.24–8.29
SMAC	Reflotron®	0.952	1.024	0.998	0.996	140.2	135.6	78	0.923–0.970	−0.94–3.36
e) Results of decentralised determinations in comparison with "reference" laboratories										
SMAC + Hitachi	Reflotron® (region A* + L**)	0.967	−5.45	0.870	0.757	93.9	87.7	251	0.879–1.055	−12.4–2.67
After outlier rejection		0.914	−1.068	0.950	0.902	94.3	84.4	244	0.838–1.001	−8.31–5.71
Linear regression		0.771	11.65			94.3	84.4	244		

* Amsterdam ** Leeuwarden

the means for the serum samples are not significantly different and the means for the plasma samples are significantly different. Figure 1 shows the scatter plot and the regression line for all serum and plasma samples.

Differences between machines

We performed a series of replicate creatinine assays with EDTA plasma and blood on the Reflotrons® 1 and 2 in the central laboratory to check differences between the machines and specimens (tab. 2a). The EDTA plasmas were prepared from EDTA blood by centrifugation. It is noted that differences between two machines do exist, that EDTA blood gives higher results than its corresponding plasma, and that the coefficients of variation of the blood determinations are higher than those of the plasma measurements.

Tab. 2. Machine characteristics as exemplified by
(a) replicate assays of patient samples on two instruments, and
(b) between day coefficient of variation with control sera.

a) Imprecision within run (patient samples)

Sample material	Reflotron® 1			
	Mean	SD	CV	N
EDTA-plasma 1	80.9	2.7	3.3	15
EDTA-blood 1	86.3	6.8	7.9	15
EDTA-plasma 2	77.3	2.9	3.8	15
EDTA-blood 2	78.1	5.5	7.1	15
Sample material	Reflotron® 2			
	Mean	SD	CV	N
EDTA-plasma 1	81.8	2.7	3.3	15
EDTA-blood 1	95.4	11.0	11.6	15
EDTA-plasma 2	78.0	2.0	2.6	15
EDTA-blood 2	88.2	9.0	10.5	15

b) Imprecision between days (control sera)

Control serum	Mean	SD	CV	N
Lot nr. 161171	212.8	9.53	4.48	47
Lot nr. 166507	247.8	7.43	3.00	48

Reproducibility

Reproducibility was checked on the Reflotron® 1 with two lots of Precinorm U control serum. Analyses were done three times a day during 16 days, and the results as shown in table 2b demonstrate a coefficient of variation of 3% and 4.5%.

Different batches of strips

Batch to batch comparison was done on the Reflotron® 1, using 41 heparin plasma patient samples and the two batches of strips. The results are shown in table 1b and figure 2. Based on the confidence interval ($p < 0.05$) for slope and intercept the batches are not different. Based on the *Student*'s test the means of the patient samples tested with the two batches are different ($p < 0.01$).

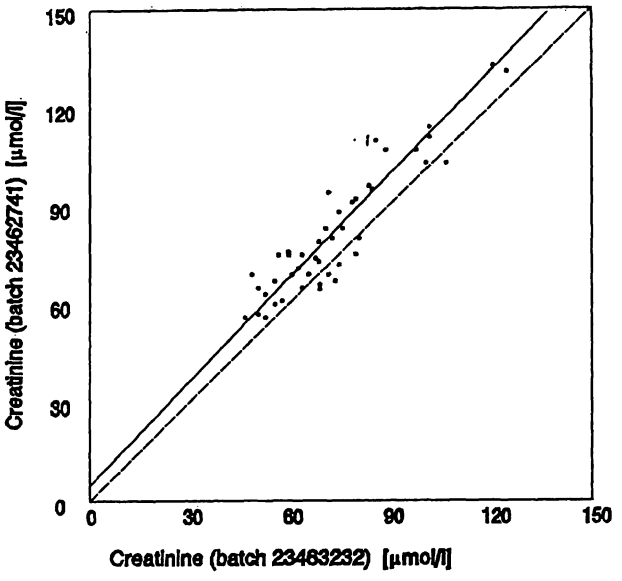

Fig. 2. Comparison of batches of creatinine reagent using the Reflotron®.

Different sample materials

The results of comparison of several sample materials used with different methods in shown in table 1c. Reflotron® experiments were done with the Reflotron® 1 from the previous tests. In this experiment no great differences were noticed between EDTA blood and plasma (fig. 3). Heparin plasma shows higher

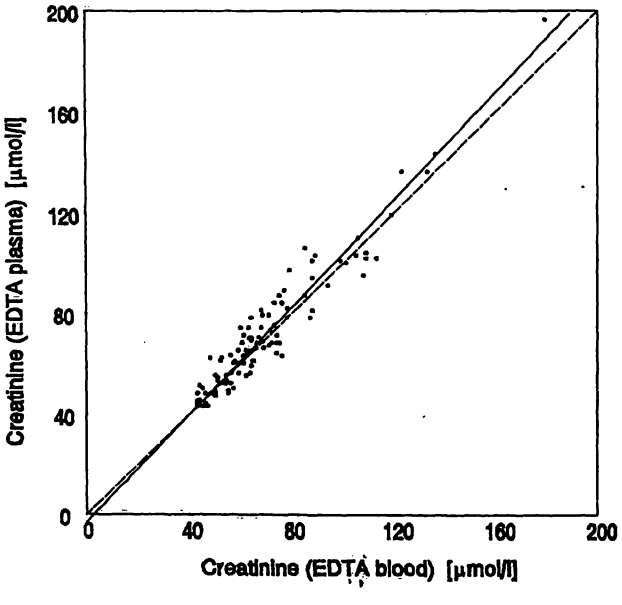

Fig. 3. Comparison of EDTA blood and plasma samples using the Reflotron®.

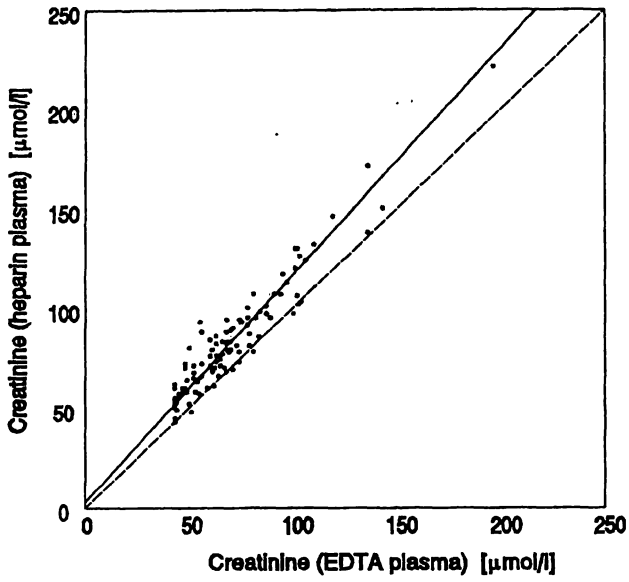

Fig. 4. Comparison of EDTA plasma and heparin plasma using the Reflotron®.

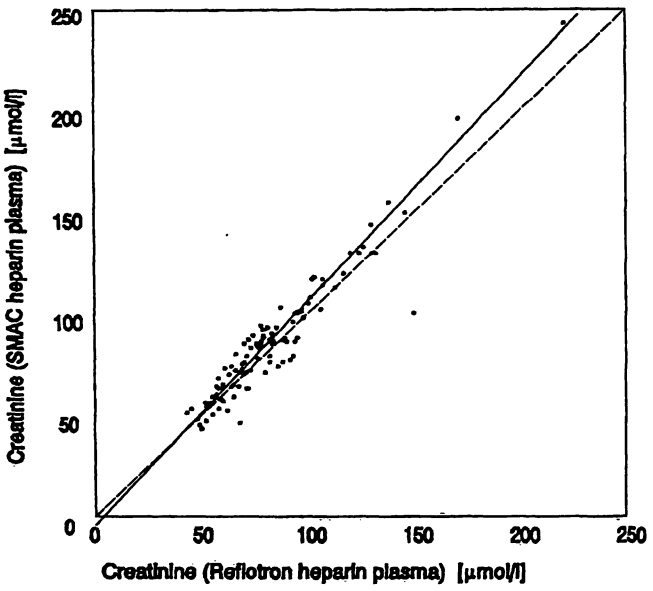

Fig. 5. Comparison of heparin plasma on SMAC and Reflotron®.

results than EDTA plasma (fig. 4). The Reflotron® gives lower results than the SMAC using heparin plasma (fig. 5).

Different methodologies

We compared the laboratory "wet chemistry" (SMAC) and the Reflotron® method with the reference method for creatinine (tab. 1d and figs. 6, 7, 8), using heparin plasma. Our reference method showed higher creatinine results.

Interferences

Glucose up to a concentration of 32 mmol/l did not influence either of the creatinine determinations used

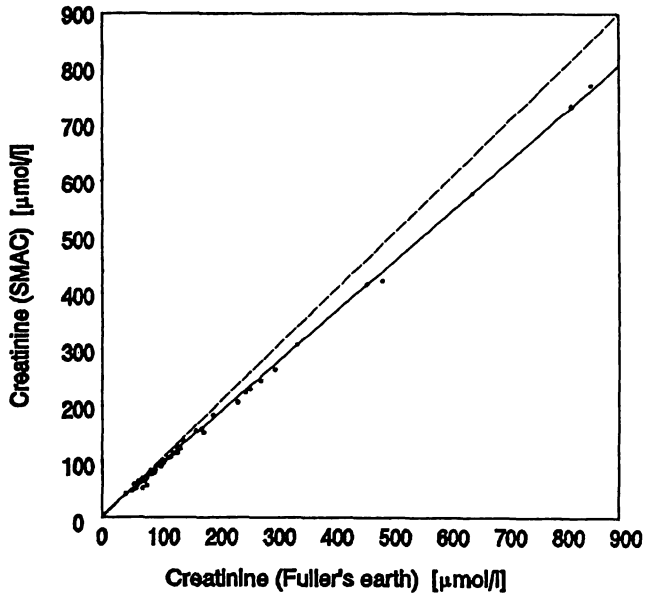

Fig. 6. Comparison *Fuller*'s earth and SMAC creatinine.

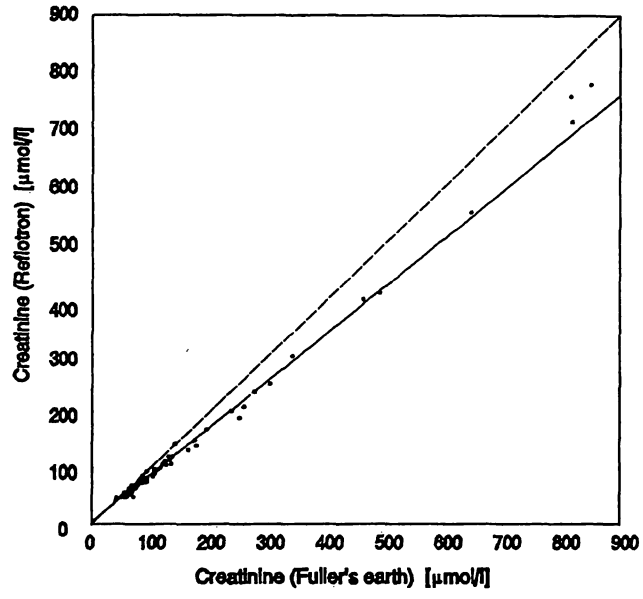

Fig. 7. Comparison *Fuller*'s earth and Reflotron® creatinine.

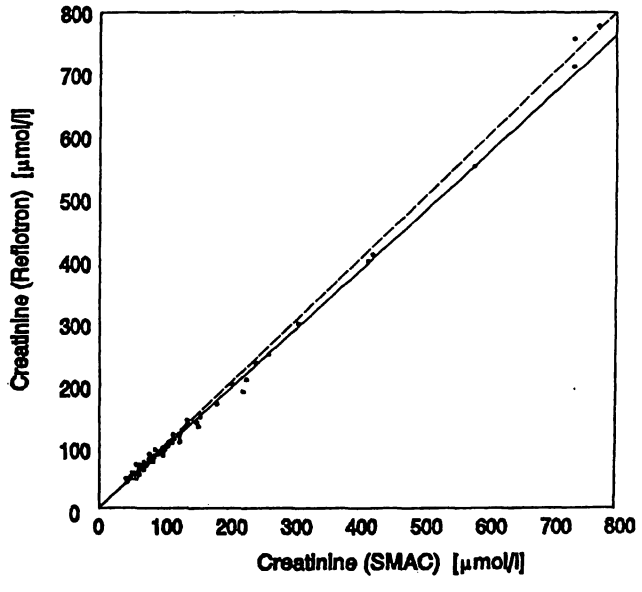

Fig. 8. Comparison SMAC and Reflotron® creatinine.

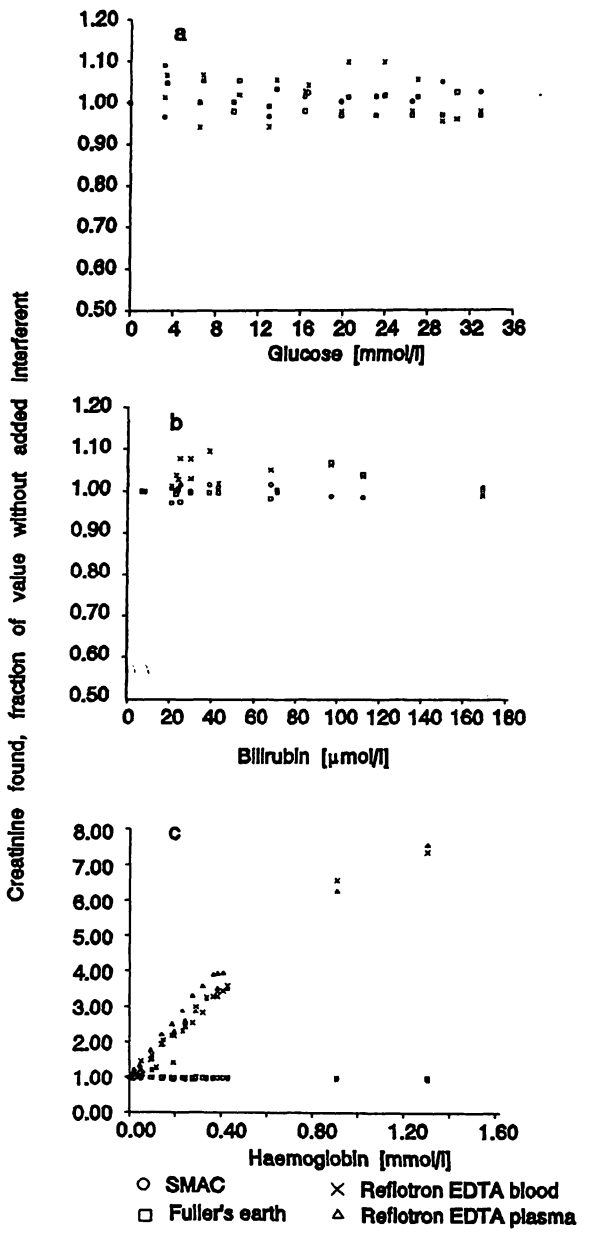

Fig. 9. Interfering substances: glucose, bilirubin and free haemoglobin.
a) glucose [mmol/l], ○ SMAC, □ *Fuller*'s earth, x Reflotron
b) bilirubin [µmol/l], ○ SMAC, □ *Fuller*'s earth, x Reflotron
c) free haemoglobin [mmol/l], ○ SMAC, □ *Fuller*'s earth, x Reflotron EDTA blood, △ Reflotron EDTA plasma

(fig. 9). Neither the Reflotron® creatinine method, nor the other methods showed any interference from bilirubin up to a concentration of 170 µmol/l (fig. 9). The Reflotron® creatinine method gave falsely elevated results when the haemoglobin concentration was above 0.05 mmol/l. With a haemoglobin concentration of 0.1 mmol/l the creatinine was raised by 50% (fig. 9); neither the *Fuller*'s earth technique, nor the SMAC method showed deviations from the original creatinine value.

Determinations in general practitioners offices

After having established the quality of the creatinine determination per se on the Reflotron®, we determined the quality of the results as performed by non-skilled personnel in doctors' offices. Table 1e shows the results of the creatinine determinations in EDTA blood performed first in a general practitioners office, followed by an analysis in serum by one of the laboratories. The results are given for all samples determined in the period of one year. The scattergram of the sample data and the difference plot are represented in figure 10. Using the EP9-P guideline we found 7 outliers (marked with black squares in the scatterplot and black dots in the difference plot in fig. 10) in the set of 251 sample data. In all these 7 cases the general practitioners found the data in conflict with this expectation of the creatinine value and asked for the "reference" laboratory result.

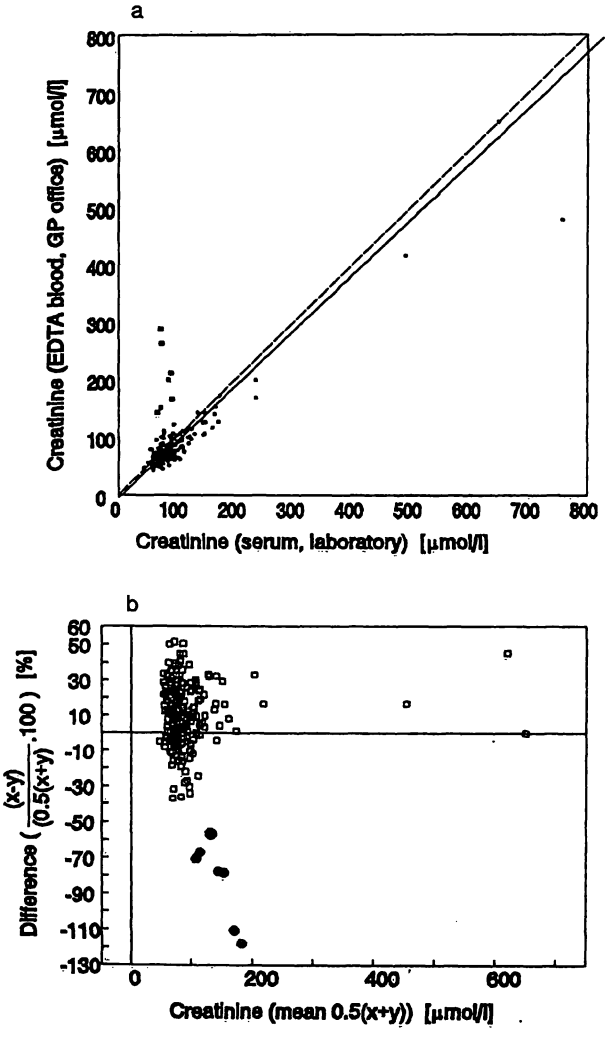

Fig. 10. Scattergram and difference plot of creatinine results from general practitioners (GP) and "reference" laboratory.

Discussion

As already stated the evaluation of the determination of creatinine on the Reflotron® is part of a greater study concerning the quality of tests in doctor's offices. All determinations performed by general practitioners were repeated as soon as possible in two hospital laboratories designated as "reference" laboratories. Although the general practitioners used EDTA-blood, since one sample then would be sufficient for both "dry chemistry" and haematology testing, we choose to exchange serum samples between the general practitioners offices and the laboratories. At the start of this study we had to be sure that no significant differences existed between the two "reference" laboratories. As table 1a and figure 1 show, there are small but irrelevant differences between the two laboratories using different methodologies for the creatinine determination: in the set of 119 data pairs we detected one outlier. Based on the linear regression equation, the difference between the laboratory values at a concentration of 140 µmol/l is 1.4 µmol/l. This concentration was chosen since it is the cut-off value used by the general practitioners in their daily practices as the trigger value for further action: the detected difference has no relevance for medical decision making.

The difference found between plasma and serum samples can possibly be explained by the effect of heparin plasma samples on the kinetic *Jaffé* reaction used on the Hitachi. Using EDTA plasma, the two different Reflotron® 74F machines gave the same results for mean and standard deviation. When EDTA blood samples were used, differences in the mean and standard deviation were found (tab. 2a); since the two machines were used in parallel for the determinations with the same samples there can be no effect of sample storage. We have no explanation for these differences, but we note that *Blijenberg* (9) also found an instrument variation and questioned the instruments' calibration.

The results obtained for the control sera (tab. 2b) fall within the range reported by the manufacturer. The coefficients of variation are in the same range (3–4.5%) as those found for plasma samples in the previous experiment. When whole blood is used, the coefficient of variation increases to 7.2–11.6% (tab. 2a). *Blijenberg* (9) reported coefficients of variation of the same magnitude. A possible explanation for the increase in coefficients of variation is the influence of variable haematocrit values on the effective sample volume.

Table 1b gives a comparison of two batches of Reflotron® creatinine reagent strips for heparin plasma samples. According to the confidence intervals, slope and intercept of the regression equation do not differ significantly from 1.00 and 0.0, respectively (7). The squared coefficient of correlation (0.854) shows that for > 85% of the data the y-value can be calculated from the x-value, using the regression equation. There is a significant difference in mean creatinine results, indicating that the calibration from batch to batch is disputable. Since this is a closed system with no facility for changing the instrument calibration, this must be seen as a drawback (9).

Table 1c shows that the means for 111 EDTA plasma and blood samples are not significantly different (fig. 3). The differences as reported in table 2a are not seen in this experiment; our impression is that the sample size plays an important role in this effect. On the Reflotron® heparin plasma samples differ from the EDTA samples from the same person (fig. 4). We have no explanation for these differences, but it is probable that EDTA influences the reagent system used in the strips; on the SMAC we never found differences between heparin plasma and EDTA plasma. In its most recent instructions Boehringer advises that heparin plasma or serum be used on the Reflotron® for the creatinine determination. The advantage is a lower coefficient of variation, but this approach is much less practicable for office testing.

In general, there is a large difference between EDTA samples and heparin samples (tab 1c). From all correlation coefficients it can be seen that the data ranges are not sufficiently extended: all samples were chosen from patients without kidney malfunction. In table 1d and figures 6 and 7 we compare the *Fuller*'s earth method using heparin plasma samples with the Reflotron® and the SMAC. The *Fuller*'s earth method gives significantly higher results than SMAC and Reflotron®. SMAC gives significantly higher results than the Reflotron®. Based on the correlation coefficients (tab. 1d) the data for the different methods correlate very well. A possible explanation for the higher results of the reference method may be found in the article of *Kanturek* et al. They showed that *Lloyd*'s reagent (*Fuller*'s earth) absorbs several *Jaffé* reacting agents (10).

Comparison between the results of the general practitioners and the "reference" laboratories shows that the overall sample set contains 7 outliers in 251 samples (fig. 10). After rejection of outliers the data range is not yet acceptable (r = 0.950). The regression data show a constant bias in this set of −1.07 µmol/l and a proportional bias of −8.5%. At a cut off concentration of 140 µmol/l the Reflotron® value would be 119.6 µmol/l (based on lineair regression). As we

demonstrated (fig. 9), interference by haemoglobin, even at a low concentration, may be responsible for the outliers. When working with whole blood samples haemolysis is not noticed, and haemoglobin concentration that are too low to be noticed with the naked eye in plasma or serum are quite able to influence the results. Interference by glucose and bilirubin was found to be negligible under normal circumstances. The difference plot shows a large scatter at values around 100 μmol/l and suggests a concentration-related difference.

Although the creatinine test on the Reflotron® gives biased results it may be applicable in the general practitioners office as long as the general practitioner is aware of its limitation. He should have a good communication with a "reference" laboratory for the necessary training, quality assurance and repetition of unexpected results (11). The general practitioner is in general very capable of distinguishing normal results from outliers. Thus, in our experiment we let the general practitioners predict the results of the determinations performed in their respective offices. In the case of outliers the prediction was nearly always in agreement with the result of the "reference" laboratory. The advice of the manufacturer to use serum or plasma severely limits the use of the creatinine test on the Reflotron® in general practitioners offices. The necessity for an extra centrifugation step diminishes its use in direct patient care and requires the investment in an expensive laboratory centrifuge. Although we are aware of the drawbacks (12), the general practitioners interested in signs of kidney malfunction might use the determination of urea on the Reflotron®, since this assay can be performed with whole blood.

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Dr. Jan L. S. Dols
Academisch Medisch Centrum
Laboratorium voor
Algemene Klinische Chemie
Meibergdreef 9
NL-1105 AZ Amsterdam
The Netherlands